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Dielectric properties of microvillous cells simulated by three-dimensional finite-element method

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Abstract

Most of biological cells have microvilli on their surfaces, which significantly influence their dielectric properties. The complex permittivity of a cubical system containing a spherical cell model with cylindrical projections was calculated over a frequency range of 10 kHz to 100 MHz using three-dimensional finite-element method. The spectra of the complex permittivity consisted of low- and high-frequency relaxation processes which were respectively attributed to the polarization of the membranes covering the projections and the spherical body. Conventional analysis based on the spherical shell model was applied to the simulated spectra to discuss effects of cell surface morphology on the electric parameters estimated for the plasma membrane and the cytoplasm.

Keywords: Interfacial polarization; Dielectric spectroscopy; Biological cell; Microvillus; Simulation.

1. Introduction

Biological cells are polarized in electric fields owing to interfacial polarization, which behavior has been studied by dielectric spectroscopy (DS) and the electrokinetic methods of dielectrophoresis (DEP) and electrorotation (ER). DS measures the complex relative permittivity ε^* of a cell suspension as a function of the frequency f of the applied ac field. Complex relative permittivity is defined as $\varepsilon^* = \varepsilon - j\kappa/\omega\varepsilon_0$, where ε is relative permittivity, κ electric conductivity, $\omega = 2\pi f$, ε_0 the permittivity of vacuum and $j^2 = -1$. On the other hand, DEP and ER respectively measure the force and torque that a single cell experiences in heterogeneous ac fields and rotating fields. The force and torque are related to the polarizability or the Clausius-Mossotti factor K of the cell. The spectra of ε^* and K have been usually analyzed by theories based on the spherical shell model that is a sphere covered with a thin shell to estimate the electric parameters of cell components such as membrane capacitance C_m and cytoplasmic conductivity κ_i [1-7]. For a spherical cell with a smooth surface, C_m is the specific membrane capacitance defined by $C_m = \varepsilon_m \varepsilon_0 / d$ and the values of 6-8 mF m⁻² have been obtained for cells with smooth surfaces like erythrocytes [8-13]. However, larger C_m -values have been obtained for many cultured cells, being mainly due to the presence of

membrane-bound projections that are extensions of the plasma membrane [14-22]. Irimajiri et al. [14] studied dielectric properties of basophil leukaemia cells whose volume was changed by osmotic perturbation, and clearly demonstrated that the estimated value of C_m changing with the cell radius R correlated to the “microvilation” or the surface roughness of the cells. Similar studies were made with mouse myeloma and hybridoma cells [15] and murine erythroleukaemia cells [16] by ER, suggesting that the cell total membrane capacitance C_t defined by $C_t = 4\pi R^2 C_m$ was kept constant during osmotic perturbation, i.e., C_t was independent of R . Further, the correlation between the value of C_m and the “microvilation” was found for cells responding to apoptosis-inducers and toxicants [17-22]. In contrast to the experimental studies, however, there are few theoretical studies about the effects of the cell surface morphology on the dielectric properties of cells.

For simple cell models such as spherical and ellipsoidal shell models, we can use analytical equations that relate the electric and morphological parameters of the cell components to the dielectric properties of cells and cell suspensions. For cells possessing complex structures, however, analytical equations are not available, and therefore numerical simulation techniques are required. Recently, various numerical techniques have been developed to simulate dielectric properties of biological cells, enabling us to deal with more realistic cell models [23-37].

This paper is concerned with numerical simulation of the dielectric properties of a cell model that emulates the morphological changes of microvilli during osmotic perturbation. Microvilli are most common membrane-bound projections of a few hundreds nanometers in diameter [38]. The length and number of microvilli change in response to environmental stimuli and cellular conditions such as osmotic perturbation, apoptosis and differentiation. In the simulation, a microvillous cell is modeled as a sphere with cylindrical projections. Electric potential distributions in a cubic box containing the cell model in a medium are numerically solved by the three-dimensional finite-element method (FEM), providing the ε^* of the system as a function of f . Since experimental ε^* spectra have been commonly analyzed using the spherical shell model irrespective of the presence of microvilli, the same analysis is applied to the simulated spectra to discuss relationships between the estimated values of C_m and κ_i and the cell surface morphology.

2. Methods

2.1. Cell Models

A microvillous cell is modeled as a sphere of radius R_s with n_p cylindrical projections of radius r_p and length h (Fig. 1). To simplify the morphological change of the cell during osmotic perturbation, it is assumed that the projections of a constant value of n_p increase in h with decreasing the cell volume V_c while keeping the total cell surface area S_c constant. S_c and V_c are approximately given by Eqs. (1) and (2), respectively, and S_c is equal to that of a sphere of radius R_0 without projection.

$$S_c = 2\pi(2R_s^2 + n_p r_p h) = 4\pi R_0^2, \quad (1)$$

$$V_c = \frac{4}{3}\pi R_s^3 + n_p \pi r_p^2 h. \quad (2)$$

The surface area ratio S_p of the projections to the whole cell becomes $S_p = \pi r_p^2 (r_p^2 + 2r_p h) / (4\pi R_0^2)$.

The cell model is placed at the center of a cubic box of $L \times L \times L$, whose top (at $z=+L/2$) and bottom (at $z=-L/2$) correspond to electrodes. Since the cell model is symmetrical about each of the vertical planes of $x=0$ and $y=0$, the domain subjected to numerical calculation can be confined to a quarter of the cubic box (Fig. 1A). The quarter is also symmetrical about the horizontal plane of $z=0$, and therefore, when voltages of +1 V and -1 V are respectively applied to the top and bottom electrodes, the middle horizontal plane (at $z=0$) becomes an equipotential surface of 0 V. This further allows us to confine the numerical calculation to a half of the quarter.

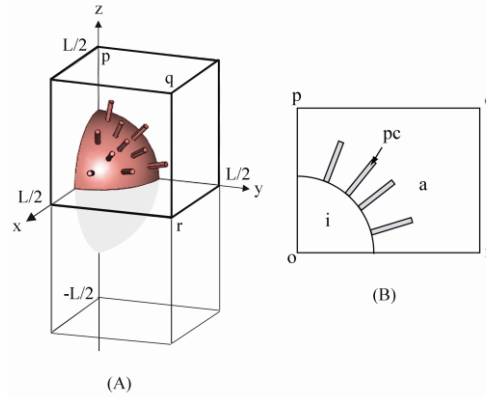


Fig. 1 (A) A cell model with cylindrical projections in a cubic box. The eighth part of the cubic box, which is rimmed with thick lines, is used for actual numerical simulation because of symmetrical geometry. The illustration corresponds to model *b* with $R_s=4 \mu\text{m}$, $r_p=0.15 \mu\text{m}$ and $n_p=80$ in a cube of $L=16 \mu\text{m}$. (B) A cross-section through points *p*, *q* and *r* in the cell model is used for cross-sectional views of the electric potential distributions shown in Fig. 6. Point *o* is the origin of the coordinate, and subdomains *pc*, *i* and *a* indicate the projection core, cytoplasm and external medium, respectively.

2.2. Numerical calculation

Numerical calculation was made by FEM using Comsol Multiphysics with an AC/DC Module (COMSOL AB) in quasi electrostatic and time harmonics modes over a frequency range from 1 kHz to 1 GHz. Biological cells possess thin plasma membranes, which require extra fine meshes and thus considerable computation tasks. To solve the problem, the “thin-layer” approximation that deals with the membrane as an interface of zero thickness was adopted in a previous paper [37]. The same technique was used for this calculation. We consider subdomains *i*, *a* and *pc* that respectively correspond to the cell interior, the external medium and the projection core (see Fig.1)). The subdomains *i*, *a* and *pc* have complex relative permittivities ϵ_i^* , ϵ_a^* and ϵ_{pc}^* , respectively. Both interfaces between the subdomains *i* and *a* (interface *i-a*) and between the subdomains *pc* and *a* (interface *pc-a*) have the specific complex capacitance of $\epsilon_0 \epsilon_m^* / d$, where *d* is the membrane thickness and ϵ_m^* is the complex relative permittivity of the membrane. As described in section 2.1, numerical calculation was performed for the eighth part of the model system, where distributions of

electric potential ϕ were solved under the following boundary conditions. (i) For the four sides of the cubic domain, electric flux density is zero. (ii) The top and the bottom of the cubic domain have potentials of 1 and 0 V, respectively. (iii) The interfaces i - a and pc - a have potential differences and respectively have electric flux densities D

$$D = -n_i \cdot \varepsilon_i^* \varepsilon_0 \nabla \phi_i = n_a \cdot \varepsilon_a^* \varepsilon_0 \nabla \phi_a = \frac{\varepsilon_m^* \varepsilon_0}{d} (\phi_a - \phi_i) , \quad (3)$$

and

$$D = -n_{pc} \cdot \varepsilon_{pc}^* \varepsilon_0 \nabla \phi_{pc} = n_a \cdot \varepsilon_a^* \varepsilon_0 \nabla \phi_a = \frac{\varepsilon_m^* \varepsilon_0}{d} (\phi_a - \phi_{pc}) , \quad (4)$$

where n_i , n_a and n_{pc} are outward normal unit vectors to the boundaries of subdomains i , a and pc , and ϕ_i , ϕ_a and ϕ_{pc} are the electric potentials of subdomains i , a and pc at the interfaces. (iv) The interface pc - i has no potential difference and holds for continuity of electric flux density as

$$-n_{pc} \cdot \varepsilon_{pc}^* \varepsilon_0 \nabla \phi_{pc} = n_i \cdot \varepsilon_i^* \varepsilon_0 \nabla \phi_i . \quad (5)$$

The free charge on the top boundary of the cube is calculated by integrating electric flux density over the boundary. The free charge and the potential difference between the top and the bottom boundaries provide the complex capacitance C^* of the cube of $L/2 \times L/2 \times L/2$, which is simply converted to the effective complex relative permittivity ε^* as $\varepsilon^* = C^* / (\varepsilon_0 L/2)$.

2.3. Determination of dielectric relaxation parameters

The frequency dependence of the ε^* obtained by numerical simulation was represented by

$$\varepsilon^* = \varepsilon_h + \frac{\Delta \varepsilon_h}{1 + (jf / f_{ch})^{\beta_h}} + \frac{\Delta \varepsilon_l}{1 + (jf / f_{cl})^{\beta_l}} + \frac{\kappa_l}{j2\pi f \varepsilon_0} , \quad (6)$$

where ε_h is the high-frequency limit of relative permittivity, $\Delta \varepsilon$ the relaxation intensity, f_c the characteristic frequency, β the Cole-Cole parameter ($0 < \beta \leq 1$), κ_l the low-frequency limit of conductivity, and subscripts h and l of $\Delta \varepsilon$, f_c and β are referred to the high- and low-frequency relaxation processes. The relaxation parameters ε_h , $\Delta \varepsilon_h$, f_{ch} , β_h , $\Delta \varepsilon_l$, f_{cl} , β_l and κ_l were determined by fitting Eq. (6) to the simulated ε^* spectra.

3. Results and discussion

3.1. Dielectric spectra of suspensions of cells with projections

Numerical simulation has been carried out using a cell model that is a sphere with cylindrical projections. To emulate dielectric behavior of microvillous cells in osmotic perturbation, the following conditions and geometric parameter values were adopted. (i) The cell surface area S_c is constant irrespective of changes in cell volume V_c and is equal to that of a sphere of $R_0 = 5 \mu\text{m}$ in radius ($S_c = 314.2 \mu\text{m}^2$). (ii) The number n_p and radius r_p of the projections are respectively fixed at

80 and 0.15 μm . (iii) The radius R_s of the spherical body and the length h of the projections are both changed with V_c . The geometric parameters of representative cell models are listed in Table 1. The volume fraction P_g of the cell model occupied in a cubic box of $L=16 \mu\text{m}$ is calculated by $P_g=V_c/L^3$. The equivalent cell radius R_{eq} was calculated from the cell volume V_c assuming a corresponding sphere as $R_{\text{eq}}=(3V_c/4\pi)^{1/3}$.

Table 1 Geometric parameters of representative cell models.

Models	$R_s/\mu\text{m}$	$h/\mu\text{m}$	$V_c/\mu\text{m}^3$	P_g	$R_{\text{eq}}/\mu\text{m}$
a	5.0	0	523.6	0.1278	5.00
b	4.4	0.94	362.1	0.0884	4.42
c	4.0	1.50	276.6	0.0675	4.04
d	3.5	2.13	191.6	0.0468	3.58

For sake of simplicity, numerical simulation was first carried out for the case of $\varepsilon_i^*=\varepsilon_{\text{pc}}^*$, using the following parameter values relevant to mammalian cells as: $\varepsilon_a=\varepsilon_i=\varepsilon_{\text{pc}}=80$, $\kappa_a=1 \text{ S m}^{-1}$, $\kappa_i=\kappa_{\text{pc}}=0.5 \text{ S m}^{-1}$, $\varepsilon_m=5$, $\kappa_m=0 \text{ S m}^{-1}$ and $d=5 \text{ nm}$. Simulation for the case of $\varepsilon_i^*\neq\varepsilon_{\text{pc}}^*$ will be discussed in section 3.2. Figure 2 shows frequency dependence of the relative permittivity ε' ($\varepsilon'=\varepsilon$) and loss factor ε'' of a cubic system containing one of cell models *a-d*. The value of ε'' was calculated by subtracting the low-frequency limit of conductivity κ_l as $\varepsilon''=(\kappa-\kappa_l)/(2\pi f\varepsilon_0)$. The dielectric spectra for a spherical model without projection (model *a*) showed dielectric relaxation with a single relaxation time, being in good agreement with those calculated from the Pauly-Schwan (PS) theory based on the spherical shell model [6]. On the other hand, cell models with projections (models *b-d*) showed broader spectra containing two relaxation processes, which were well represented by Eq. (6) to provide the dielectric relaxation parameters of the low-frequency (LF) relaxation ($\Delta\varepsilon_l, f_{\text{cl}}, \beta_l$) and the high-frequency (HF) relaxation ($\Delta\varepsilon_h, f_{\text{ch}}, \beta_h$).

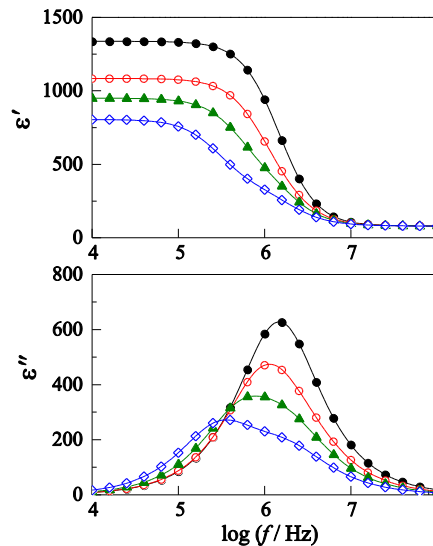


Fig. 2 The relative permittivity ε' and loss factor ε'' of a cubic system containing a cell model as a function of frequency f . Data points (\bullet), (\circ), (\blacktriangle) and (\diamond) respectively correspond to models a - d given in Table 1. Curves are the best-fit ones calculated from Eq. (6).

The dielectric relaxation parameters were plotted for the equivalent cell radius R_{eq} in Figs. 3-5. It should be noted that data points at $R_{eq}=4.42$ and $4.71 \mu\text{m}$ included large errors because the values of f_{cl} and f_{ch} were close to each other. In Fig. 3, $\Delta\varepsilon$ ($\Delta\varepsilon=\Delta\varepsilon_l+\Delta\varepsilon_h$) and $\Delta\varepsilon_h$ decrease with decreasing R_{eq} while $\Delta\varepsilon_l$ slightly changes. The ratio $\Delta\varepsilon_l/\Delta\varepsilon$ is, however, proportional to the surface area ratio S_p of the projections to the whole cell (see the inset of Fig. 3), suggesting the contribution of the projections to the LF relaxation. The f_{cl} and f_{ch} are separated by one decade at R_{eq} of about $3 \mu\text{m}$, and approach to each other with increasing R_{eq} and merges into the characteristic frequency of the model without projection at $R_{eq}=5 \mu\text{m}$ (Fig. 4). The Cole-Cole parameter β determines the shape of dielectric relaxation. The decrease in β_h with decreasing R_{eq} indicates broadening of the HF relaxation, while the values of β_l are almost unity, i.e., the Debye type relaxation for the LF relaxation (Fig. 5). At low R_{eq} , in addition to the broadening of the HF relaxation, a large difference between f_{cl} and f_{ch} results in broadening of the whole spectrum.

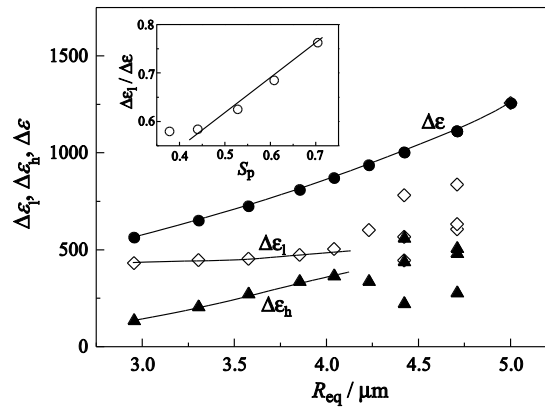


Fig. 3 Dielectric relaxation intensities $\Delta\varepsilon_l$ (\diamond), $\Delta\varepsilon_h$ (\blacktriangle), and $\Delta\varepsilon$ (\bullet) as a function of equivalent cell radius R_{eq} . Inset: $\Delta\varepsilon_l/\Delta\varepsilon$ plotted against the relative surface area S_p of the projections to the whole cell. Curves are a guide to eye.

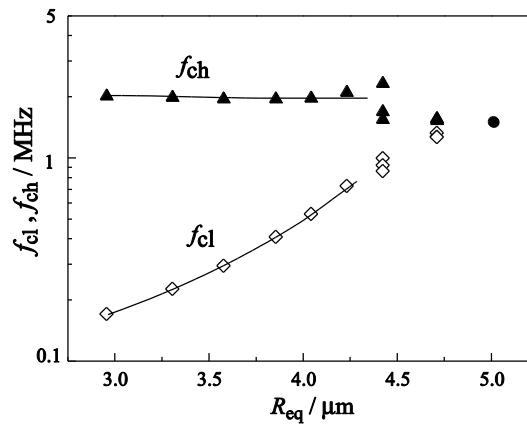


Fig. 4 Characteristic frequencies f_{cl} (\diamond) and f_{ch} (\blacktriangle) as a function of equivalent cell radius R_{eq} . The point (\bullet) at $R_{eq}=5 \mu\text{m}$ refers to the model without projection (model a). Curves are a guide to eye.

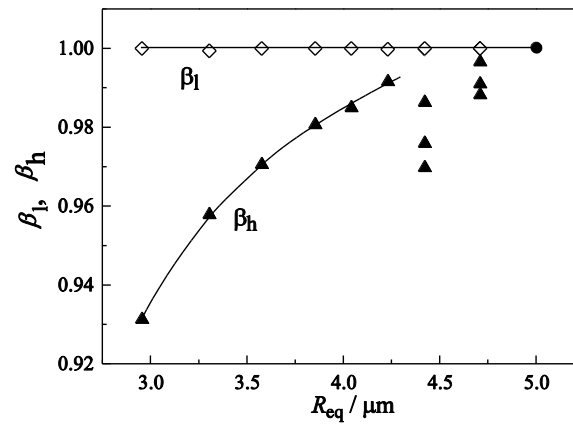


Fig. 5 Cole-Cole parameters β_1 (◇) and β_h (▲) as a function of equivalent cell radius R_{eq} . The point (●) at $R_{eq}=5 \mu\text{m}$ refers to the model without projection. Curves are a guide to eye.

The LF and HF relaxation processes might be due to the membrane polarization of the projections and the spherical body, respectively. To assign the two relaxation processes, we examine electric potential distributions in the system at different frequencies. Figure 6 shows the distributions of the real part of electric potential for model *d*. The insides of the projections and the spherical body both have a constant potential below 100 kHz and are clearly distinguished from the surroundings, indicating that the membranes of the projections and the spherical body are both fully polarized. At 1 MHz, where the LF relaxation diminishes, the projections become invisible and the projection membrane is less polarized. Finally the spherical body disappears at 10 MHz, where the HF relaxation almost diminishes. This behavior suggests that the LF and HF relaxation processes are related to the membrane polarization of the projections and the spherical body, respectively.

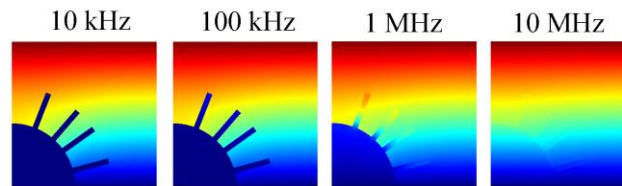


Fig. 6 Distributions of the real part of electric potential at the cross-section through points *p*, *q* and *r* shown in Fig. 1. The data are the same as those used for the dielectric spectra of model *d* in Fig. 2. The frequency of the applied ac field is shown at the top of each panel.

3.2. Effects of the electric properties of the projection core

The core of a microvillus contains actin filaments that run in parallel bundle along its length. Thus, the electric properties of the projection core could be different from the cytoplasm. Here, we examine the effects of the electric properties of the projection core on the dielectric spectra of the cell suspension. Figure 7 shows the dielectric spectra obtained by changing the conductivity κ_{pc} of the projection core while the relative permittivity ϵ_{pc} of the projection core is fixed at 80. The LF

relaxation shifted to a lower frequency side with decreasing κ_{pc} , whereas the HF relaxation was not influenced by κ_{pc} . When the value of ε_{pc} was changed between 160 and 40, there was little influence on the dielectric spectra (data are not shown). The results also support the assignment that the LF and HF relaxation processes are attributed to the polarization of the projections and the spherical body, respectively.

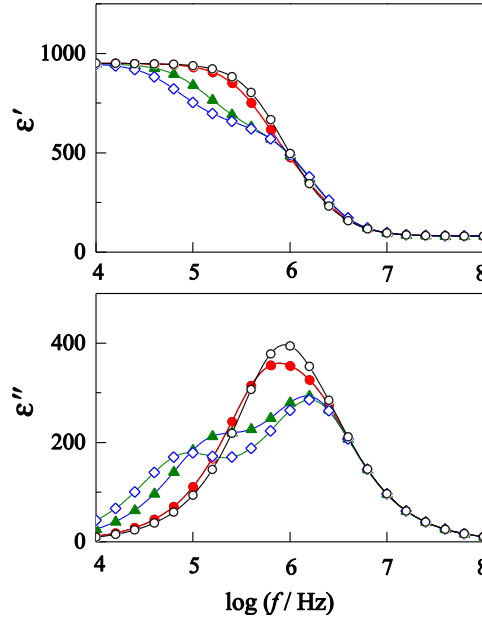


Fig. 7 The relative permittivity ε' and loss factor ε'' as a function of frequency f , which have been simulated for model b when the projection core has different conductivities in S m^{-1} : 1 (\circ), 0.5 (\bullet), 0.1 (\blacktriangle) and 0.05 (\diamond). The other parameter values are the same as in Fig. 2

3.3. Apparent membrane capacitance and cytoplasmic conductivity

Dielectric spectra of spherical cell suspensions have been analyzed using the spherical shell model irrespective of cell surface morphology. In the analysis, the volume fraction P of cells (or the relative volume occupied by cells in a cell suspension), the membrane capacitance C_m and the cytoplasmic conductivity κ_i are determined from the dielectric relaxation parameters of the dielectric spectra. The C_m of a spherical cell with a smooth surface is the specific membrane capacitance defined by $C_m = \varepsilon_m \varepsilon_0 / d$, while an apparent value of C_m is obtained for a cell with a rough surface. The relationship between the value of C_m and the degree of membrane folding has not been clearly understood. In addition, the question is raised whether the surface morphology influences the estimations of P and κ_i . To answer the questions, the same analysis was applied to the simulated dielectric spectra.

When cells hold for the conditions of $d/R \ll 1$ and $\kappa_m \ll \kappa_a \approx \kappa_i$, the PS theory based on the spherical shell model predicts dielectric relaxation of a single relaxation time. The dielectric relaxation parameters κ_i , $\Delta\varepsilon$, f_c and κ_h (the high-frequency limit of conductivity) are related to P , C_m and κ_i [3, 6] as:

$$P = \frac{2(1 - \kappa_i / \kappa_a)}{2 + \kappa_i / \kappa_a}, \quad (7)$$

$$C_m = \Delta\epsilon \frac{\epsilon_0(2+P)^2}{9PR}, \quad (8)$$

$$\kappa_i = \left[\frac{9P}{2\pi f_c \Delta\epsilon \epsilon_0 (2+P)^2} - \frac{1-P}{\kappa_a(2+P)} \right]^{-1}, \quad (9)$$

$$\kappa_i = \kappa_a \frac{(2+P)\kappa_h - 2(1-P)\kappa_a}{(1+2P)\kappa_a - 2(1-P)\kappa_h}. \quad (10)$$

Analysis with Eqs. (7)-(10) was carried out for the dielectric spectra simulated for the case of $\epsilon_i^* = \epsilon_{pc}^*$ described in section 3.1. Figure 8 shows the values of P calculated from Eq. (7) plotted against those of P_g calculated from the geometry of the cell model. The values of P were in good agreement with those of P_g , suggesting that Eq. (7) is applicable to microvillous cells at volume fractions below at least 0.13 and that the estimation of P is insensitive to the cell surface morphology.

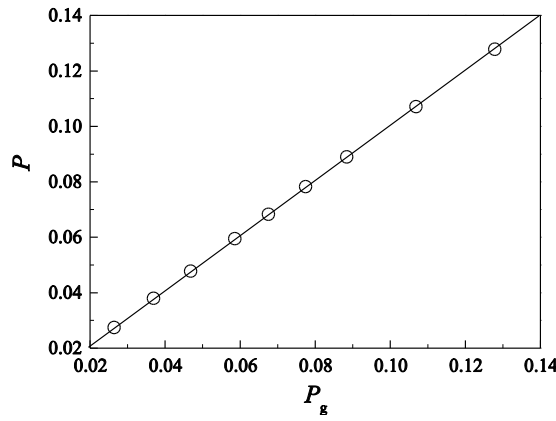


Fig. 8 The volume fraction P calculated from Eq. (7) as a function of the volume fraction P_g that is the relative volume occupied by the cell model in the cubical system. A line with a slope of unity is shown.

The values of C_m were calculated from Eq. (8) with the values of R and P . The choice of the value of R is, however, arbitrary, although, in experiment, the value of R is determined by optical microscopy. Here, the values of R_{eq} and R_s were used for R . Figure 9 shows double logarithmic plots of the estimated values of C_m against R . Linear relations were found, whose slopes were -2.27 with $R=R_{eq}$ and -2.16 with $R=R_s$. As a first approximation, therefore, C_m is inversely proportional to R^2 as $C_m \approx C_{m0}(R_0/R)^2$, where C_{m0} is the specific membrane capacitance of the spherical cell (without projection) of radius R_0 . Using this relation, the cell total membrane capacitance C_t becomes $C_t = 4\pi R^2 C_m \approx 4\pi R_0^2 C_{m0}$. Hence, C_t is independent of R , which relation accounts for the experimental results that C_t remained constant irrespective of changes in R with the medium osmolality [15, 16]. In other words, if the value of C_{m0} is given, the value of C_t provides the total surface area of the cell. For the value of C_{m0} , we may adopt a value of 6-8 mF m⁻² obtained for erythrocyte membranes with smooth surfaces.

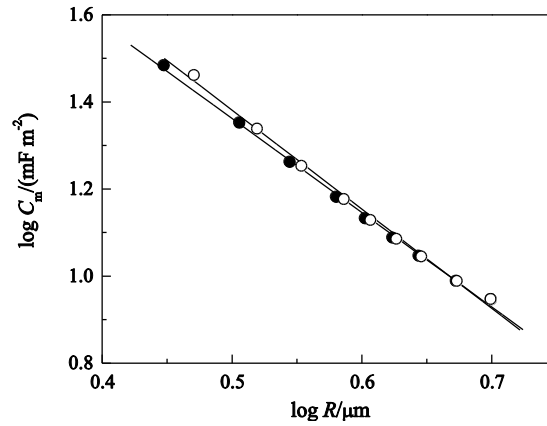


Fig. 9 The membrane capacitance C_m estimated by Eq. (8) as a function of the cell radius R . Data points (\circ) and (\bullet) are calculated with $R=R_{eq}$ and R_s , respectively. Solid lines are regression lines.

The cytoplasmic conductivity κ_i was estimated from the values of f_c and κ_h using Eqs. (9) and (10), respectively (Fig. 10). The value of f_c was approximately determined assuming a single relaxation process, although the simulated dielectric spectra contained two relaxation processes. The value of κ_h was obtained at 100 MHz, where the conductivity almost leveled off. The values of κ_i estimated from those of f_c seriously deviated from the true value (0.5 S m^{-1}) at low R_{eq} , whereas the estimation of κ_i from κ_h provided reasonable values over the whole range of R_{eq} . The results suggest that the estimation of κ_i from f_c includes serious errors for microvillous cells in contrast to that from κ_h .

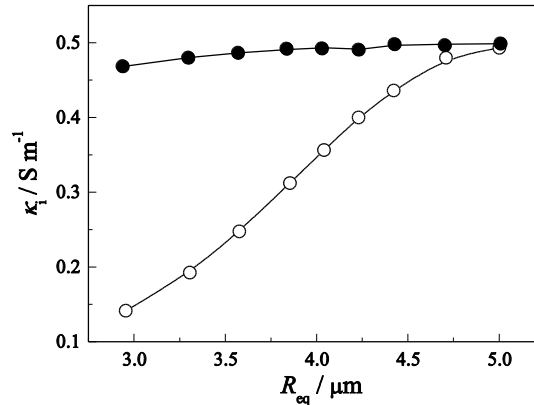


Fig. 10 Cytoplasmic conductivity κ_i as a function of effective cell radius R_{eq} . Data points (\circ) and (\bullet) are calculated from Eqs. (9) and (10), respectively.

4. Conclusion

A microvillous cell was modeled as a sphere with projections and its dielectric properties were numerically simulated by 3D-FEM. The ε^* of the cell suspension showed dielectric dispersion consisting of LF and HF relaxation processes, which were respectively attributed to the polarization of the membranes covering the projections and the spherical body. The values of C_m were obtained from the simulated spectra of ε^* by the conventional analysis based on the spherical shell model. When leaving the surface area of the cell model constant, the C_m was inversely proportional to the

square of the apparent cell radius and the cell total membrane capacitance remained constant, which was consistent with previous experimental results. The estimation of κ_i from the value of f_c in ε^* spectra included errors, and consistent values of κ_i were obtained from the value of κ_h .

The present simulation has demonstrated that the dielectric properties of cells are seriously influenced by their surface morphology. When applying the conventional spherical shell model to microvillous cells, careful considerations are required in interpreting the estimated values of electric parameters of the plasma membrane and the cytoplasm.

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